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SYNTHESIS OF 25-HYDROXY-19-NORVITAMIN D_3 ANALOGS AND THEIR ANTIPROLIFERATIVE ACTIVITIES ON PROSTATE CELLS

Midori A. Arai,[1] Ryuji Tsutsumi,[1] Hideki Hara,[1] Tai C. Chen,[2] Toshiyuki Sakaki,[3] Naoko Urushino,[4] Kuniyo Inouye,[4] and Atsushi Kittaka*,[1]

[1]Faculty of Pharmaceutical Sciences, Teikyo University, Kanagawa 199-0195, Japan [2]Boston University School of Medicine, Boston, MA 02118, USA [3]Faculty of Engineering, Toyama Prefectural University, Toyama, Japan [4]Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan e-mail address: akittaka@pharm.teikyo-u.ac.jp

Abstract – Synthesis of 25-hydroxy-19-norvitamin D_3 derivatives as prohormone type agents for anti-prostate diseases was accomplished utilizing Julia-type olefination. Synthesized compounds showed potent antiproliferative activity on an immortalized normal prostate cell line, PZ-HPV-7, which has high 1α-hydroxylase activity. Furthermore, we demonstrated that 25-hydroxy-19-norvitamin D_3 was hydroxylated at the 1α position to form 1α,25-dihydroxy-19-norvitamin D_3 by a recombinant human 1α-hydroxylase (CYP27B1) in a cell-free system.

INTRODUCTION

The biological active form of vitamin D, 1α,25-dihydroxyvitamin D_3, 1α,25$(OH)_2D_3$ (**1**), plays a major role in calcium-phosphorus homeostasis, cell differentiation and apoptosis.[1] Synthetic analogs of **1** have received much attention as potential drugs for the treatment of osteoporosis,[2] psoriasis,[3] hereditary vitamin D-resistant rickets,[4] and chemoprevention of cancer and cancer chemotherapy, especially prostate cancer, colon cancer, and breast cancer.[5] The natural hormone (**1**) is known to inhibit the proliferation and invasiveness of prostate cancer cells. However, **1** can cause hypercalcemia; therefore, the analogs that are less calcemic but exhibit potent antiproliferative activity would be attractive as therapeutic agents. It has been shown that prostate cells possess 1α-hydroxylase, (1α(OH)ase), and can convert 25-hydroxyvitamin D_3, 25$(OH)D_3$ (**2**) to **1** intracellularly to inhibit their proliferation.[5] It is also known that a

This paper is dedicated to the memory of the Emeritus Professor Kenji Koga of the University of Tokyo.

19-demethylenated analog of **1**, i.e., 1α,25-dihydroxy-19-norvitamin D_2, possesses similar pro-differentiation and antiproliferative activities as **1**.[6] Since 19-norvitamin D derivatives are known to be less calcemic than **1** when administered systemically,[7] we are interested in knowing whether 25-hydroxy-19-norvitamin D_3, 25(OH)19-norVD$_3$ (**3**) can exert potent antiproliferative activity toward prostate cells which possess 1α(OH)ase activity and therefore can be used as chemopreventive agents without causing hypercalcemic side effects. Herein, we report an improved synthesis of **3** and its diastereoisomer (**14**) by using our new coupling method utilizing Julia-type olefination,[8] and their biological activities.

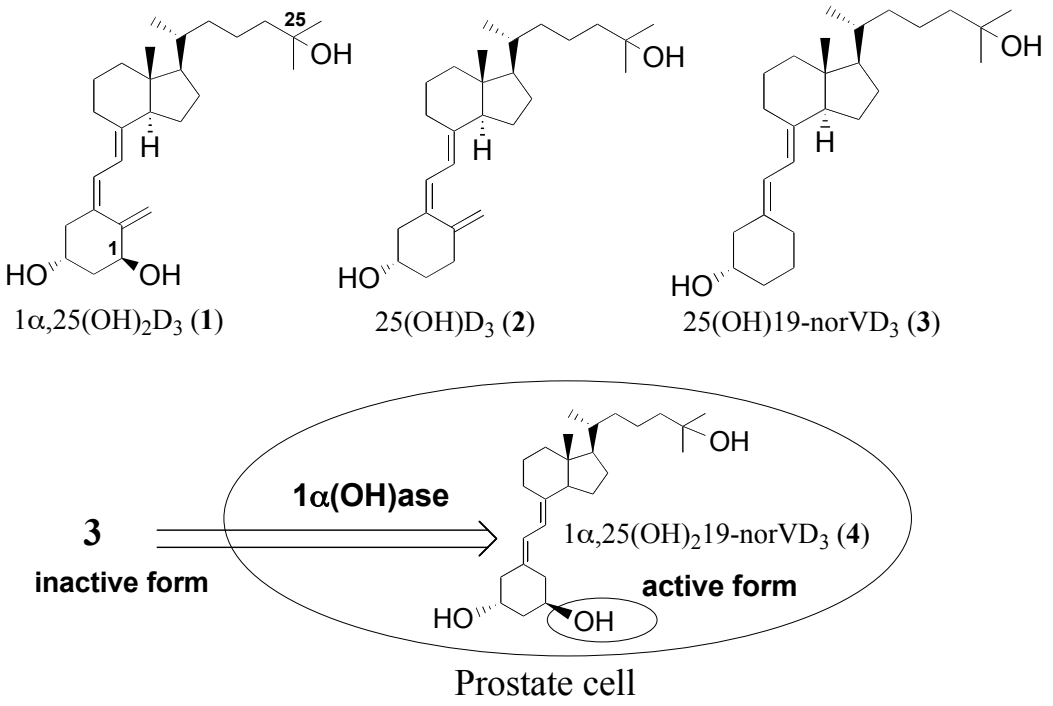
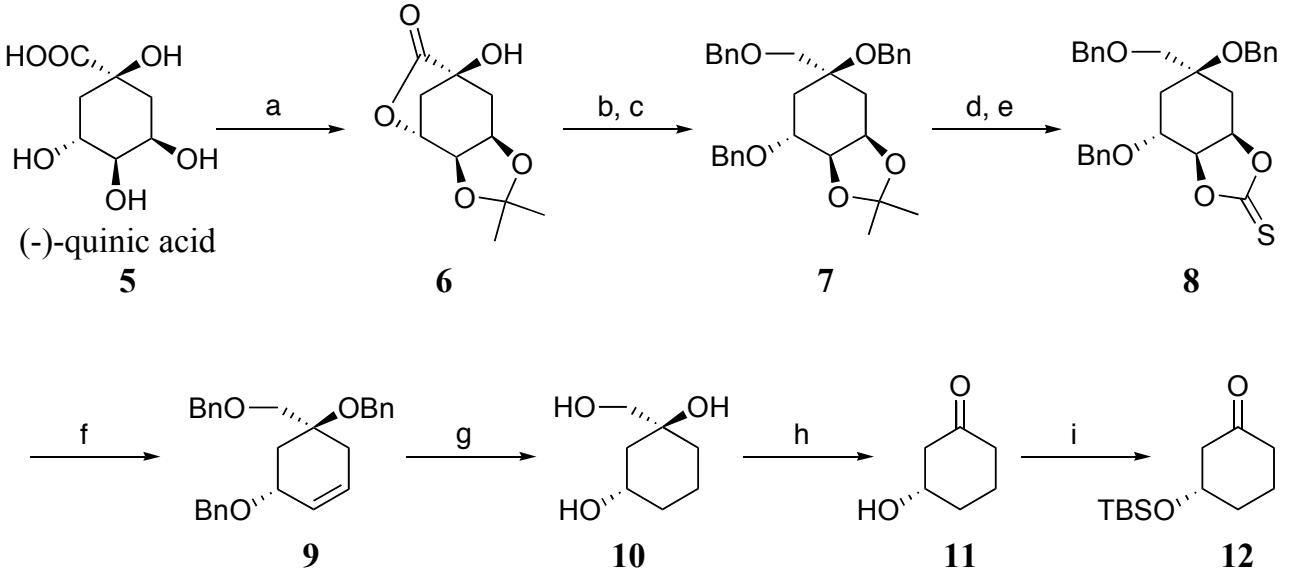


Figure 1. 25-Hydroxy-19-norvitamin D_3; Prohormone type anti-agent for prostate disease

RESULTS AND DISCUSSION

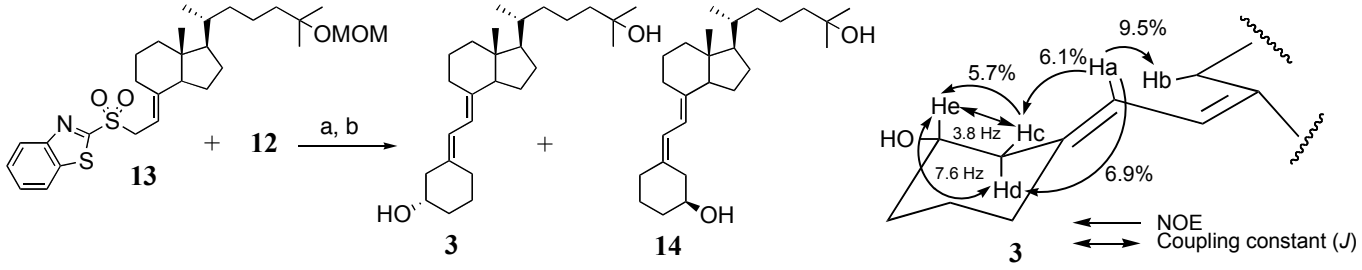
25-Hydroxy-19-nor-vitamin D_3 (**3**) was synthesized according to our new method described previously,[8,9] and its antiproliferative activity was evaluated in prostate cells. The A-ring precursor (**12**) for Julia-type olefination coupling was synthesized from (-)-quinic acid (**5**) as an enantiomeric pure form as shown in Scheme 1. After obtaining compound (**6**),[8] the triol which was obtained by sodium borohydride reduction was converted to benzyl ether (**7**). The isopropylidene group of **7** was removed under acidic conditions, and the resulting *cis*-diol was treated with bis(imidazol-1-yl)thione to give cyclic carbonothioate (**8**). By subsequent refluxing in $P(OMe)_3$,[10] the *cis*-diol unit of **8** was successfully converted to a C-C double bond to give **9**. Under hydrogenation conditions, the benzyl group was cleaved and the C-C-double bond was

reduced simultaneously to give compound (**10**), then oxidative cleavage of vicinal diol and protection of the secondary hydroxyl group by the *tert*-butyl dimethyl silyl group gave the A-ring precursor (**12**).



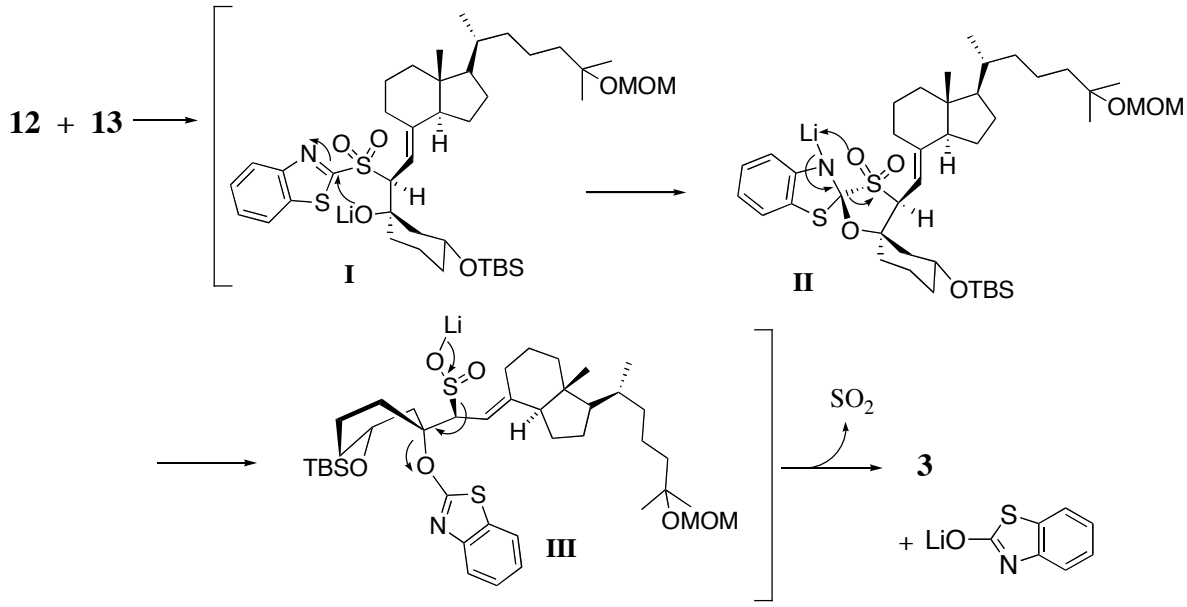
Scheme 1. Reagents and conditions: (a) 2,2-dimethoxypropane, (+)-CSA, benzene, 80%; (b) $NaBH_4$, EtOH, quant.; (c) BnBr, NaH, THF, 91%; (d) PPTS, MeOH, 92%; (e) bis(imidazol-1-yl)thione, acetone, quant.; (f) trimethyl phosphite, 86%; (g) 5% Pd-C, H_2, THF, quant.; (h) $NaIO_4$, CH_2Cl_2/H_2O, 80%; (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, 62%.

The key step of Julia-type olefination proceeded with high yields between **12** and sulfone (**13**)[8] using LiHMDS in THF at -78˚C to give the protected 19-norvitamin D_3 derivatives as a diastereomeric mixture. After partial purification, mixture was treated with (+)-CSA in MeOH to yield deprotected compounds (**3**) and 3-deoxy-1α,25(OH)$_2$-19-norvitamin D_3 (**14**) in the ration of 1 to 1 (Scheme 2). These isomers were separated by preparative TLC followed by purification with reversed-phase HPLC. From ^{1}H NMR spectroscopic experiments including NOEs, the relative configuration was determined as shown in Scheme 2.



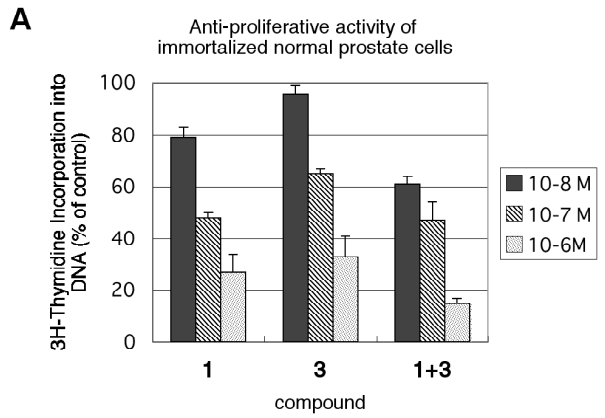
Scheme 2. Reagents and conditions: (a) LiHMDS, THF, -78C; (b) (+)-CSA, MeOH, 58% in two steps.

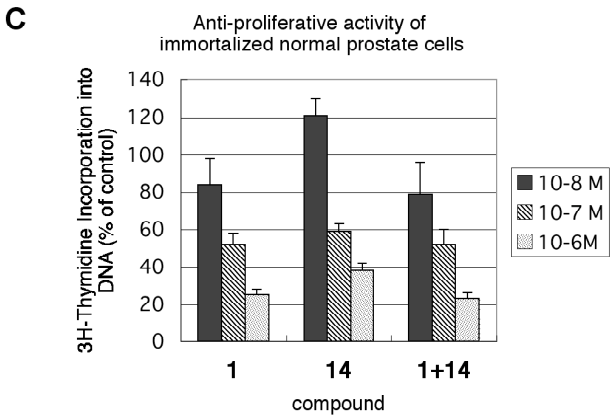
The proposed mechanism of Julia olefination for the synthesis of **3** is shown in Scheme 3 on the basis of a report by L. B. Baudin *et al.*[11] The α-lithio derivative of **13** would react with the carbonyl group of **12** to give intermediate (**I**) and lithium alkoxide. The alkoxide then reacts with the neighboring C=N group to form spiro heterocyclic intermediate (**II**). A ring-opening of the spiro heterocycle followed by a benzothiazole transfer from S to O will form the intermediate (**III**). Finally, the removal of sulfur dioxide leads to diene compound (**3**) and benzothiazolone anion.



Scheme 3. Proposed mechanism of Julia-type olefination for synthesis of **3**

Next, we evaluated the antiproliferative activities of **3** and **14** in an immortalized normal prostate cell line, PZ-HPV-7, which has high 1α(OH)ase activity. The results are summarized in Figure 2. Interestingly, both compounds showed high antiproliferative activities almost at the same level as that of the natural hormone (**1**). In the case of **3**, when the cells were treated with both **3** and the natural hormone (**1**), synergistic effect was clearly observed (graph A at 10^{-6} M). It is interesting that both **3** and **4** showed such high antiproliferative activities, even though the binding affinity of these two compounds to the vitamin D receptor (VDR) is low.[9] To evaluate a more precise molecular mechanism, we next studied the metabolism of **3** by 1α(OH)ase (CYP27B1) in an *in vitro* system (Figure 3). After incubating **3** with the *E. coli* extract containing the recombinant 1α(OH)ase (CYP27B1), the resulted mixture was analyzed by HPLC. It was shown that **3** was hydroxylated at the 1α-position to form 1α,25(OH)$_2$-19-norvitamin D$_3$ (**4**), which is known to be active. Thus, our data strongly suggest that the antiproliferative activity of **3** in prostate cells is primarily the result of its hydroxylation by endogenous 1α(OH)ase (CYP27B1) at the 1α-position leading to the formation of the active 1α,25(OH)$_2$-19-norvitamin D$_3$ compound.





B

Anti-proliferative activity on immortalized normal prostate cells [a]

Conc.	1α,25(OH)$_2$D$_3$ (**1**) (% of control)	25(OH)19-nor D$_3$ (**3**) (% of control)	**1** + **3** (% of control)
10^{-8} M	79 ± 4 [b]	96 ± 3	61 ± 3
10^{-7} M	48 ± 2	65 ± 2	47 ± 7
10^{-6} M	27 ± 7	33 ± 8	15 ± 2

[a] Cell type: PZ-HPV-7 cells, an immortalized normal prostate cell line.
[b] Data are expressed as means±SD of 11-12 determinations.

D

Anti-proliferative activity on immortalized normal prostate cells [a]

Conc.	1α,25(OH)$_2$D$_3$ (**1**) (% of control)	3-deoxy-1α,25(OH)$_2$19-nor D$_3$ (**14**) (% of control)	**1** + **14** (% of control)
10^{-8} M	84 ± 14 [b]	121 ± 9	79 ± 17
10^{-7} M	52 ± 6	59 ± 4	52 ± 8
10^{-6} M	25 ± 3	38 ± 4	23 ± 3

[a] Cell type: PZ-HPV-7 cells, an immortalized normal prostate cell line.
[b] Data are expressed as means±SD of 6 determinations.

Figure 2. Antiproliferative activity of **3** and **14** on immortalized normal prostate cells

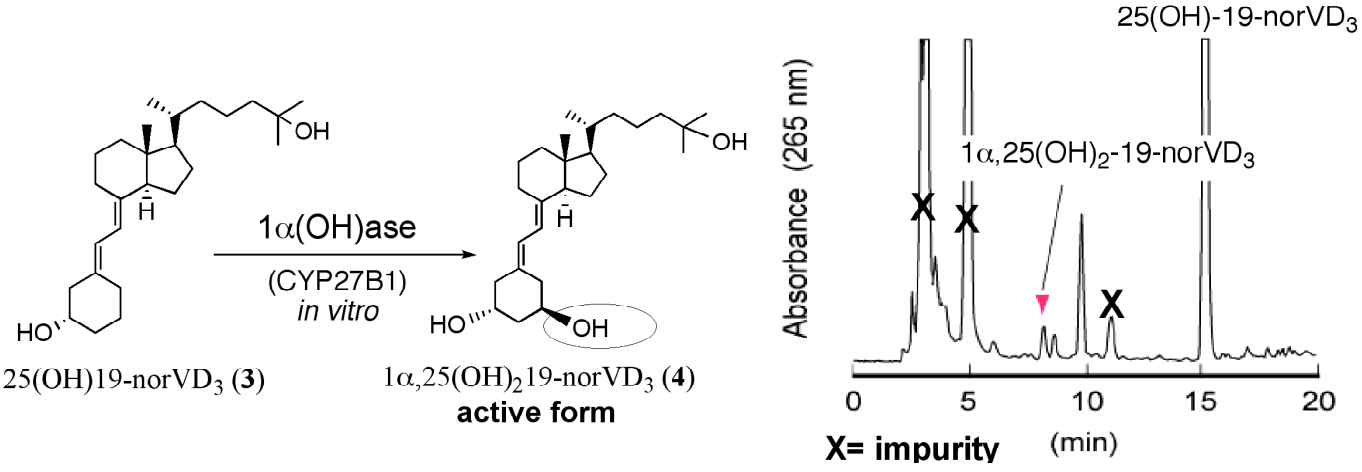


Figure 3. Metabolic activation of **3** to **4** by 1α(OH)ase

CONCLUSION

We have synthesized the A-ring deoxy-19-norvitamin D$_3$ derivatives, 25-hydroxy-19-norvitamin D$_3$ (**3**) and 3-deoxy-1α,25(OH)$_2$-19-norvitamin D$_3$ (**14**) by using Julia olefination, which we have reported as the new coupling method for the synthesis of 19-norvitamin D derivatives.[8] Both compounds exhibited high antiproliferative activities in the immortalized normal prostate cells at the same level as that of the

biological active form of vitamin D_3 (**1**). We also evaluated the metabolic reaction of **3** by 1α(OH)ase (CYP27B1) in cell-free system, and found that the 1α-hydroxylated **4** was produced. This is the first report that A-ring deoxy-19-norvitamin D_3 derivatives are highly active in inhibiting prostate cell growth. Precise mechanistic studies are in progress.

EXPERIMENTAL

(3a*S*,4*R*,6*R*,7a*R*)-4,6-Bis(benzyloxy)-6-(benzyloxymethyl)hexahydro-2,2-dimethylbenzo[*d*][1,3]dioxole (7). To a solution of **6** (5.83 g, 27.2 mmol) in EtOH (120 mL) was added $NaBH_4$ (3.09 g, 81.6 mmol) at 0 ºC. The reaction mixture was stirred at the same temperature for 2.5 h. The reaction was quenched by addition of acetone at 0 ºC. Evaporation of the solvent afforded a crude mixture, from which (3a*S*,4*R*,6*R*,7a*R*)-hexahydro-6-hydroxymethyl-2,2-dimethyl-1,3-benzodioxole-4,6-diol[8,12] (6.06 g) was roughly separated by silica gel short pad column chromatography (MeOH : $CHCl_3$ = 1 : 5) as a white solid in quantitative yield.

To a suspension of NaH (60% in mineral oil, 2.26g, 56.6 mmol) in THF (7 mL) was added with stirring a solution of the above triol (617 mg, 2.83 mmol) in THF (8 mL) at 0 ºC under argon. After stirring for 70 min at rt, to the reaction mixture was added benzyl bromide (5.05 mL, 42.5 mmol) at 0 ºC. The resulting mixture was stirred at rt for 22 h, and then heated at 70 ºC for 25 h. After the addition of MeOH and water to the reaction mixture at 0 ºC, the whole was extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and filtered. The solvent was removed under the reduced pressure to give a residue, from which **7** (1.26 g) was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 5) as a colorless oil in 91% yield. $[\alpha]_D^{25}$ = -21.1° (*c* = 0.94, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (3H, s), 1.38 (3H, s), 1.51 (1H, dd, *J* = 13.7, 11.8 Hz), 1.89 (1H, dd, *J* = 15.6, 5.1 Hz), 2.15-2.02 (1H, m), 2.34-2.39 (1H, m), 3.45 (1H, d, *J* = 9.4 Hz), 3.49 (1 H, d, *J* = 9.4 Hz), 3.93 (1H, ddd, *J* = 11.8, 7.0, 4.2 Hz), 4.06 (1H, dd, *J* = 7.0, 6.5 Hz), 4.34-4.39 (1H, m), 4.40 (1H, d, *J* = 11.0 Hz), 4.53 (2H, s), 4.56 (1H, d, *J* = 11.0 Hz), 4.62 (1H, d, *J* = 12.0 Hz), 4.74 (1H, d, *J* = 12.0 Hz), 7.21-7.36 (15H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.9, 28.1, 31.0, 35.2, 64.6, 71.7, 73.4, 73.6, 75.1, 75.3, 76.2, 80.3, 108.6, 127.0, 127.3, 127.5, 127.6, 127.8, 128.0, 128.2, 128.3, 138.0, 138.7, 139.2; IR (film) 3031, 2984, 2932, 2865, 1497, 1455, 1366, 1240, 1053, 739, 698 cm^{-1}; MS m/z 473 (M^+ - Me); HRMS calcd for $C_{30}H_{33}O_5$ 473.2328, found 473.2337.

(3a*S*,4*R*,6*R*,7a*R*)-4,6-Bis(benzyloxy)-6-(benzyloxymethyl)hexahydrobenzo[*d*][1,3]dioxole-2-thione (8). A solution of **7** (1.24 g, 2.54 mmol) in MeOH (25 mL) was treated with PPTS (64 mg, 254 μmol) at rt under argon. After refluxing at 70 ºC for 23 h, to the reaction mixture was added sat. aq. $NaHCO_3$ and evaporated. The whole was extracted with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and filtered. Evaporation of the solvent afforded a crude mixture, from which

(1*R*,2*R*,3*R*,5*S*)-3,5-bis(benzyloxy)-5-(benzyloxymethyl)cyclohexane-1,2-diol (1.05 g) was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 2) as a colorless oil in 92% yield. $[\alpha]_D^{26}$ = -1.0° (*c* = 1.03, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (1H, dd, *J* = 14.1, 11.6 Hz), 1.64 (1H, dd, *J* = 15.0, 3.4 Hz), 2.30 (1H, ddd, *J* = 15.0, 3.3, 3.2 Hz), 2.50 (1H, ddd, *J* = 14.1, 3.8, 3.8 Hz), 3.42 (1H, d, *J* = 9.9 Hz), 3.46 (1H, d, *J* = 9.9 Hz), 3.52 (1H, dd, *J* = 9.3, 3.3 Hz), 3.72 (1H, ddd, *J* = 11.6, 9.3, 4.4 Hz), 4.03-4.04 (1H, m), 4.26 (1H, d, *J* = 10.6 Hz), 4.47 (1H, d, *J* = 10.6 Hz), 4.52 (2H, s), 4.72 (1H, d, *J* = 12.0 Hz), 4.77 (1H, d, *J* = 12.0 Hz), 7.14-7.16 (2H, m), 7.27-7.41 (13H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.7, 36.5, 64.9, 70.4, 72.3, 73.4, 74.1, 74.6, 76.3, 79.8, 127.5, 127.6, 127.7, 127.7, 127.9, 128.4, 128.4, 128.4, 137.6, 137.7, 138.5; IR (film) 3461, 3063, 3031, 2924, 2867, 1495, 1455, 1091, 737, 698 cm^{-1}; MS m/z 357 (M^+ - Bn); HRMS calcd for $C_{21}H_{25}O_5$ 357.1702, found 357.1697.

To a solution of the above diol (84 mg, 188 μmol) in acetone (2 mL) was added bis(imidazol-1-yl)thione (168 mg, 940 μmol) at rt under argon. After refluxing at 60 ºC for 2 days, the reaction mixture was evaporated. The whole was diluted with CH_2Cl_2, washed with 1 M aq. HCl, sat. aq. Na_2CO_3, and brine. The organic layer was dried over magnesium sulfate and filtered. Removal of the solvent afforded a residue, from which **8** (94 mg) was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 3) as pale yellow oil in quantitative yield. $[\alpha]_D^{25}$ = -24.7° (*c* = 1.02, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (1H, dd, *J* = 14.0, 11.9 Hz), 2.00 (1H, dd, *J* = 16.1, 5.0 Hz), 2.24 (1H, ddd, *J* = 14.0, 4.7, 2.1 Hz), 2.64 (1H, ddd, *J* = 16.1, 2.9, 2.6 Hz), 3.46 (1H, d, *J* = 9.3 Hz), 3.54 (1H, d, *J* = 9.3 Hz), 4.04 (1H, ddd, *J* = 11.9, 7.2, 4.7 Hz), 4.34 (1H, d, *J* = 10.8 Hz), 4.50 (1H, d, *J* = 10.8 Hz), 4.52 (2H, s), 4.57 (1H, d, *J* = 11.5 Hz), 4.70 (1H, d, *J* = 11.5 Hz), 4.83 (1H, dd, *J* = 7.6, 7.2 Hz), 5.11 (1H, ddd, *J* = 7.6, 5.0, 2.9 Hz), 7.23-7.38 (15H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 34.5, 64.9, 72.3, 73.5, 73.8, 74.2, 75.1, 80.5, 84.9, 127.4, 127.5, 127.6, 127.8, 127.8, 127.9, 128.3, 128.4, 128.4, 137.4, 137.4, 137.9, 190.9; IR (film) 3063, 3031, 2932, 2863, 1497, 1455, 1273, 1121, 1096, 739, 698 cm^{-1}; MS m/z 490 (M^+), 399 (M^+ - Bn); HRMS calcd for $C_{29}H_{30}O_5S$ 490.1814, found 490.1812.

(3*R*,5*R*)-3,5-Bis(benzyloxy)-5-benzyloxymethylcyclohexene (9). Compound (**8**) (70 mg, 143 μmol) was treated with trimethyl phosphite (2 mL) at rt under argon. After refluxing at 110 ºC for 2 days, the reaction mixture was diluted with ether, washed with 10% aq. NaOH, water, and brine. The organic layer was dried over sodium sulfate, filtered, and evaporated. The residue was diluted with toluene, and maleic anhydride (240 mg) was added. The resulting mixture was refluxed at 110 ºC for 30 min, and then 10% aq. NaOH was added at rt. After being stirred for 15 min, the reaction mixture was diluted with ether, washed with 10% aq. NaOH, water, and brine. The organic layer was dried over sodium sulfate and filtered. Removal of the solvent afforded a residue, from which **9** (51 mg) was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 5) as a colorless oil in 86% yield. $[\alpha]_D^{25}$ = +66.3° (*c* = 1.02, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.81 (1H, dd, *J* = 12.9, 8.8 Hz), 2.23-2.30 (1H, m),

2.33-2.38 (1H, m), 3.55 (2H, s), 4.26-4.32 (1H, m), 4.49 (2H, s), 4.54 (1H, s), 4.54 (1H, s), 4.58 (1H, s), 4.59 (1H, s), 5.69-5.74 (1H, m), 5.88 (1H, d, J = 10.3 Hz), 7.21-7.36 (15H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.8, 34.7, 64.5, 70.4, 72.4, 73.3, 74.5, 76.4, 125.8, 127.1, 127.2, 127.4, 127.5, 127.6, 128.1, 128.1, 128.2, 128.3, 138.2, 138.7, 139.3; IR (film) 3063, 3031, 2861, 1655, 1495, 1455, 1071, 912, 737, 693 cm^{-1}; MS m/z 323 (M^+ - Bn); HRMS calcd for $C_{21}H_{23}O_3$ 323.1647, found 323.1649.

(1*S*,3*S*)-1-Hydroxymethylcyclohexane-1,3-diol (10). A solution of **9** (511 mg, 1.23 mmol) in dry THF (13 mL) was treated with Pd-C (5%, 787 mg) at rt under H_2 atmosphere. The reaction mixture was stirred for 5 days, diluted with MeOH, and filtered. Evaporation of the solvent afforded a residue, from which **10** (196 mg) was purified by silica gel column chromatography (MeOH : ethyl acetate = 1 : 10) as a white solid in quantitative yield. mp 114.0-115.0 ˚C (AcOEt-Hexane); $[\alpha]_D^{21}$ = +7.4˚ (c = 0.93, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18-1.28 (4H, m), 1.61-1.71 (5H, m), 1.98-2.02 (2H, m), 3.47 (2H, s), 4.00 (1H, dddd, J = 8.8, 8.8, 4.4, 4.4 Hz); MS m/z 146 (M^+), 115 (M^+ - CH_2OH), 97 (M^+ - CH_2OH - H_2O); HRMS calcd for $C_7H_{14}O_3$ 146.0943, found 146.0949; *Anal.* calcd for $C_7H_{14}O_3$•1/7H_2O: C, 56.52; H, 9.68, found: C, 56.47; H, 9.95.

(*S*)-3-Hydroxycyclohexanone (11). To a solution of **10** (193 mg, 1.32 mmol) in CH_2Cl_2 (7 mL), $NaIO_4$ (847 mg, 3.96 mmol) in water (7 mL) was added dropwise at 0 ºC. After stirring for 1.5 h at rt, the mixture was extracted with CH_2Cl_2 and ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. Evaporation of the solvent afforded a crude mixture, from which **11** (121 mg) was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 1) as a colorless oil in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66-1.82 (2H, m), 1.99-2.13 (2H, m), 2.33 (2H, dd, J = 7.1, 6.3 Hz), 2.41 (1H, dd, J = 14.1, 7.8 Hz), 2.67 (1H, dd, J = 14.1, 4.1 Hz), 4.20 (1H, dddd, J = 7.8, 7.8, 4.1, 3.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 32.8, 40.9, 50.4, 69.7, 209.8; MS m/z 114 (M^+), 97 (M^+ - OH); HRMS calcd for $C_6H_{10}O_2$ 114.0681, found 116.0681.

(*S*)-3-(*tert*-Butyldimethylsiloxy)cyclohexanone (12). To a stirred mixture of **11** (115 mg, 1.01 mmol) and 2,6-lutidine (410 μL, 3.54 mmol) in dry CH_2Cl_2 (5 mL) was added TBSOTf (487 μL, 2.12 mmol) at 0 ºC under argon. The resulting mixture was stirred for 4 h, and the reaction was quenched by addition of water. After extraction with CH_2Cl_2, the organic layer was washed with brine, dried over magnesium sulfate, and filtered. Removal of the solvent afforded a residue, from which **12** (144 mg) was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 9) as a colorless oil in 62% yield.

$[\alpha]_D^{25}$ = -5.6˚ (c = 1.02, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.87 (9H, s), 1.63 - 1.78 (2H, m), 1.86-1.92 (1H, m), 2.03-2.12 (1H, m), 2.27-2.32 (2H, m), 2.38 (1H, dd, J = 13.9, 6.8 Hz), 2.53 (1H, dddd, J = 13.9, 3.7, 1.1, 1.1 Hz), 4.17 (1H, dddd, J = 6.8, 6.8, 3.7, 3.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.8, -4.8, 18.0, 20.5, 25.7, 33.4, 41.1, 50.8, 70.2, 209.7; IR (film) 2955, 2857, 1715, 1462, 1362, 1254,

1034, 835, 777 cm^{-1}; MS m/z 228 (M^+), 215 (M^+ - Me), 171 (M^+ - ^{t}Bu); HRMS calcd for $C_{12}H_{24}O_2Si$ 228.1545, found 228.1546.

(1*S*,5*E*,7*E*)-19-Nor-9,10-secocholesta-5,7-diene-1,25-diol (4) and (1*S*,5*Z*,7*E*)-19-Nor-9,10-secocholesta-5,7-diene-1,25-diol (13). To a solution of **12** (172 mg, 323 μmol) in dry THF (800 μL) was added LiHMDS (1 M solution in THF, 303 μL, 303 μmol) at -78 ºC under argon. After stirring at the same temperature for 65 min, a solution of **13** (46 mg, 202 μmol) in dry THF (800 μL) was added dropwise to the mixture. After stirring for 3 h, the reaction mixture was quenched by addition of sat. aq. NH_4Cl, and the aqueous layer was extracted with ether. The organic layer was washed with brine, dried over sodium sulfate, and filtered. After evaporation of the solvent, the crude mixture was applied on silica gel column chromatography (ethyl acetate: *n*-hexane = 1 : 30) to give the crude protected vitamin D mixture (75 mg in total). To a solution of the protected vitamin D mixture (75 mg) in dry MeOH (1.5 mL) was added (+)-CSA (64 mg, 274 μmol) at 0 ºC under argon. After stirring at rt for 20 h, the reaction mixture was diluted with ethyl acetate. The resulting mixture was washed with sat. aq. $NaHCO_3$ and brine, dried over magnesium sulfate, and evaporated. Purification by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 1) gave a mixture of **3** and **14** (45 mg, 58% in two steps) as white solid. Separation of **3** and **14** was conducted by using a silica gel preparative TLC (ethyl acetate : *n*-hexane = 1 : 3) to yield **3** and **14** as white powders, respectively.

(1*S*,5*E*,7*E*)-19-Nor-9,10-secocholesta-5,7-diene-1,25-diol (3). $[\alpha]_D^{21}$ = +61.4° (*c* = 0.70, $CHCl_3$); UV (EtOH) λ_{max} 243, 252, 261 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.94 (3H, d, *J* = 6.3 Hz), 1.03-1.09 (1H, m), 1.18-1.66 (19H, m), 1.22 (6H, s), 1.71-1.77 (1H, m), 1.85-1.91 (2H, m), 1.98-2.01 (2H, m), 2.11-2.17 (2H, m), 2.35 (1H, ddd, *J* = 13.5, 6.9, 4.5 Hz), 2.50 (1H, dd, *J* = 12.9, 3.8 Hz), 3.80 (1H, dddd, *J* = 7.6, 7.6, 3.8, 3.8 Hz), 5.82 (1H, d, *J* = 11.2 Hz), 6.13 (1H, d, *J* = 11.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.1, 18.8, 20.8, 22.3, 23.1, 23.5, 27.7, 27.9, 28.8, 29.2, 29.4, 34.7, 36.1, 36.4, 40.5, 44.4, 45.7, 46.0, 56.3, 56.5, 70.1, 71.1, 115.6, 120.6, 135.7, 141.7; IR (film) 3389, 2942, 2870, 1618, 1468, 1377, 1055, 758 cm^{-1}; MS m/z 388 (M^+), 370 (M^+ - H_2O); HRMS calcd for $C_{26}H_{44}O_2$ 338.3341, found 338.3333.

(1*S*,5*Z*,7*E*)-19-Nor-9,10-secocholesta-5,7-diene-1,25-diol (14). $[\alpha]_D^{27}$ = +73.8° (*c* = 0.84, $CHCl_3$); UV (EtOH) λ_{max} 243, 251, 261 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 (3H, s), 0.94 (3H, d, *J* = 6.6 Hz), 1.02-1.10 (1H, m), 1.20-1.67 (18H, m), 1.22 (6H, s), 1.75-1.82 (1H, m), 1.88-1.95 (2H, m), 1.98-2.03 (2H, m), 2.07-2.10 (2H, m), 2.13-2.18 (1H, m), 2.78-2.88 (2H, m), 3.69 (1H, dddd, *J* = 8.6, 8.6, 4.3, 4.3 Hz), 5.85 (1H, d, *J* = 11.2 Hz), 6.21 (1H, d, *J* = 11.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.0, 18.8, 20.8, 22.3, 23.5, 24.3, 27.7, 28.8, 29.2, 29.4, 35.3, 36.1, 36.3, 36.4, 37.9, 40.5, 44.4, 45.7, 56.3, 56.5, 70.3, 71.1, 115.4, 120.3, 135.9, 141.9; MS m/z 388 (M^+), 370 (M^+ - H_2O); HRMS calcd for $C_{26}H_{44}O_2$ 338.3341, found 338.3332.

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